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# Connection between sulfur metabolism and Hyn hydrogenase in *Thiocapsa roseopersicina* BBS

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The purple sulfur phototrophic bacterium, *Thiocapsa roseopersicina* BBS preferably utilizes sulfur compounds as electron donors and carbonate as inorganic carbon source for growth. Its sulfur metabolism is similar to that of *Allochromatium vinosum* but several variances could be recognized. *T. roseopersicina* contains several enzymatic routes for oxidation sulfur compounds, such as the modified Sox cycle which has an indispensable role in the assimilation thiosulfate, few sulfide oxidoreductases and the Dsr complex which is likely linked to sulfur oxidation. All these processes release electrons which – in principle - might be converted to $H_2$ via the hydrogenases and/or nitrogenase of the strain.

Hydrogenases are metalloenzymes capable of oxidation of molecular hydrogen and proton reduction. *Thiocapsa roseopersicina* BBS has four active NiFe hydrogenases. Hox1, Hox2 are cytoplasmic $NAD^+$ reducing hydrogenases, while the other two enzymes (Hyn, Hup) are bound to the membrane. The Hup and Hox1 hydrogenases are likely connected to the central quinone pool.

The main electron transport routes to/from the hydrogenases are not fully understood. In order to disclose these metabolic pathways, we cultivated single hydrogenase containing strains, in the presence of various kind of electron donors and the amounts of $H_2$ and various sulfur compounds were followed.

Hyn hydrogenase can produce hydrogen in the absence of carbonate. Under these conditions, sodium thiosulfate could promote hydrogen evolution, while the expression level of Hyn remained the same. Therefore, the elevated $H_2$ might be derived from the more intense metabolic flux. It was also shown that the oxidation of zero-valent sulfur can donate electrons to Hyn. Under these conditions, sulfur is an exclusive electron donor for both hydrogen evolution of Hyn and hydrogen sulfide formation which are consequently competitive processes. These results suggest that Hyn hydrogenase has a role in the elimination of extra electrons released from sulfur oxidation and protection against toxic effect of sulfide. Hydrogen evolution of Hyn hydrogenase was found only under illumination Moreover, the oxidation of various sulfur compounds was also blocked in darkness, therefore the light dependency of hydrogen evolution might be an indirect consequence of the light requirement of sulfur oxidation.

Glutathione amide forms were shown to be potential redox carrier in purple sulfur bacteria. Their role was investigated in the electron transport between sulfur metabolism and Hyn hydrogenase. In the absence of glutathione amide reductase there was an elevated hydrogen evolution by Hyn which indicated a competition between glutathione amide and Hyn hydrogenase for the electrons.

Oppositely, in the presence of elemental sulfur, hydrogen addition increased the Hyn mediated hydrogen sulfide formation, thus the connection between Hyn hydrogenase and sulfur metabolism was proved to be bidirectional. The Hyn dependent hydrogen sulfide formation was not light dependent. It was also pointed out that the two electron transport subunits of HynSL -Isp12- were indispensable in this linkage.

The interrelationship of hydrogen and sulfur metabolism was clearly demonstrated at physiological level. Based on these results, an integrated – but still hypothetical – electron transport model was outlined.

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# The supramolecular organization of photosystem II in vivo studied by circular dichroism spectroscopy

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The light reactions of photosynthesis in higher plants take place in granal chloroplast thylakoid membranes, which contain chirally organized macrodomains composed of photosystem II (PSII) supercomplexes associated with light harvesting antenna complexes (LHCIIs). The physiological relevance of this hierarchic organization, which often manifest itself in semicrystalline macro-assemblies, has not been elucidated but the diversity of the supramolecular structures and their reorganizations under different conditions indicates its regulatory role. The present work focuses on the structural and functional roles of different components of LHCII-PSII supercomplexes. We used various growth conditions, influencing the protein composition, and different Arabidopsis mutants (koCP24, koCP26, koPsbW, koPsbX, dgd1), with altered organization of the membranes, and measured their circular dichroism (CD) spectra as well as their chlorophyll fluorescence kinetics to characterize the chiral macro-organization of the chromophores and the functional parameters of the membranes, respectively.

We have shown that the LHCII components play important roles in the macro-organisation of thylakoid membranes. We found that although these pigment-protein complexes themselves have only limited capacity to form ordered arrays in vivo, they can promote the

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formation of long-range chiral order of chlorophyll and carotenoid molecules, as manifested in Ψ-type CD. The role of LHCII in determining the CD is mainly exerted via organising the PSII supercomplexes into chirally organized macro-assemblies, rather than via increasing the number of interacting chromophores. PSII associated with LHCII appears to possess higher ability to form macro-domains than either the core complexes or LHCIIs on their own. Coexistence of the LHCII and PSII core in the membrane - but without coupling (by LMW proteins) - appears to result in less ordered arrays.

Our data also reveal specific functions of some of the protein and lipid compounds in the light adaptation processes of plants.

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## Role of Pituitary Adenylate Cyclase-Activating Polypeptide (PACAP) in the trigeminovascular system

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Abnormal activation of trigeminovascular system (TS) plays important role in the development of migraine, but its precise mechanism is still unknown. Recent clinical and experimental data have suggested that Pituitary Adenylate Cyclase-Activating Polypeptide-38 (PACAP-38) might contribute to the evolution of migraine-attacks. Therefore, we aimed to examine the PACAP-immunoreactivity (IR) in blood plasma of migraineurs, in interictal and ictal periods as well as in blood plasma and nerve tissues in two animal models of activated TS.

Adult Sprague-Dawley rats were got involved in the experiments. Electrical stimulation (ES) of the trigeminal ganglion (TRIG) was applied at 10 Hz, 1 mA for 30 min and in a separate group of rats 10 mg/kg dose of nitroglycerol (NTG) was injected ip. Peripheral blood samples were collected, and three brain regions, involved in migraine (caudal trigeminal nucleus-TNC; TRIG; cervical 3-5 of the spinal cord-SC) were dissected at different time-points after the stimulation of the TS.

In the clinical study 40 control subjects and 60 migraineurs were examined, selected by the criteria of the International Headache Society. Blood samples were collected from 21 migraineurs in the interictal and drug-free ictal periods.

In both case, the blood samples were taken to tubes containing EDTA and protease inhibitor, then the plasma was separated (2000 rpm 10 min 4°C). The plasma and nerve tissue samples were stored at -80°C till the PACAP radioimmunoassay measurements.

In rats the plasma PACAP-IR significantly increased 90 and 180 min after ES compared to the sham-stimulated and intact control groups. ES also evoked a remarkable elevation of PACAP level in the TNC at the 180 min time-point. In the NTG-model plasma PACAP-IR remained unchanged, but significant PACAP-IR increase was observed in the TNC 90 and 180 min following the chemical stimulation. The level of this peptide was not substantially altered in the TRIG and the SC in either model.

Significantly lower PACAP-38-IR were detected in human interictal migraine samples, than in the control group (Student's *t*-test for unpaired comparisons; $p<0,002$). Self-control comparison of PACAP-38-IR of 17 migraineurs in the ictal and interictal periods showed significant elevation during the attack (Student's *t*-test for paired comparisons; $p<0,002$).

It is concluded, that in animals the elevated PACAP-IR in the systemic circulation and/or in the TNC induced by PACAP release from both the peripheral and central terminals of the trigeminal pseudounipolar neurones.

The reduced concentration of PACAP-38 in the interictal period might be due to energy deficit. After a trigger the peptide can release from the sensory nerve terminals. The level of PACAP starts to significantly increase in the systemic circulation and induces vasodilatation, neuronal activation, sensitization, which are responsible for the initiation of pathomechanism of primary headache, like migraine.

The crucial role of PACAP in the activation mechanisms of TS is assumed. The nervous system specific examinations of PACAP can provide new perspectives to identify a new target in the therapy of migraine.

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